

## BIOLOGICAL CONTROL

Biological, Nutritional, and Histochemical Basis for Improving an Artificial Diet for *Bracon hebetor* Say (Hymenoptera: Braconidae)SANDRA R. MAGRO<sup>1,3</sup>, ALCIDES B. DIAS<sup>2</sup>, WALTER R. TERRA<sup>2</sup> AND JOSÉ R.P. PARRA<sup>1</sup><sup>1</sup>Depto. Entomologia, Fitopatologia e Zoologia Agrícola - ESALQ/USP, C. postal 9, 13.418-900, Piracicaba, SP<sup>2</sup>Depto. Bioquímica - Instituto de Química / USP, C. postal 26077, 05513-970, Butantã, São Paulo, SP<sup>3</sup>Faculdade Integrado de Campo Mourão, Rodovia BR 158, km207, 87300-970, Campo Mourão, PR

---

Neotropical Entomology 35(2):215-222 (2006)Bases Biológicas, Nutricionais e Histoquímicas para o Aprimoramento de Dieta Artificial para *Bracon hebetor* Say (Hymenoptera: Braconidae)

RESUMO - Avaliou-se a biologia de *Bracon hebetor* Say (Hymenoptera: Braconidae) criada em lagartas de 5º instar de *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) (dieta natural) e em dieta artificial. Caracterizou-se o número de ínstar, o tempo de desenvolvimento e o consumo de alimento; testes histoquímicos permitiram observar a presença/ausência de lipídios e proteínas no trato digestivo do parasitóide. Tais estudos possibilitaram detectar diferenças para o aprimoramento da dieta artificial para a criação do parasitóide. *B. hebetor* apresentou três instares larvais em ambos os substratos alimentares, contudo o ciclo do parasitóide foi prolongado quando criado em dieta artificial, devido ao alongamento das fases de larva e pupa. *B. hebetor* consumiu aproximadamente 3,8 µl de dieta artificial e, considerando o mesmo fluxo de alimentação, aqueles criados sobre o hospedeiro ingeriram 2,7 µl (proporcionalmente em relação ao tempo e ao peso) e apresentaram ganho de peso mais rápido. Análises da hemolinfa do hospedeiro natural, da dieta artificial e do tubo digestivo de larvas de *B. hebetor* indicaram a necessidade de adição de outras fontes de lipídios e proteínas na dieta artificial, buscando aproximar o sistema *in vitro* daquele representado pelas lagartas hospedeiras (sistema *in vivo*).

PALAVRAS-CHAVE: Controle biológico, ácido graxo, idiobionte, exigência nutricional, proteína

ABSTRACT - The biology of *Bracon hebetor* Say (Hymenoptera: Braconidae) reared on fifth instars of *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) (natural diet) and *in vitro* (artificial diet) was evaluated. Data on the number of instars, development time and food intake were collected, and histochemical tests were conducted to detect proteins and lipids in the parasitoid's digestive tract. The data disclosed differences that can help to improve artificial rearing of *B. hebetor*. *B. hebetor* had three instars in both diets, but the developmental time on the artificial diets was prolonged due to the increase in larval and pupal development times. Larvae grew faster on the natural host and required a lower food intake (2.7 µl) as compared to that required by the larvae feeding on the artificial diet (3.8 µl). Analysis of diet protein content and host hemolymph and the observations on the parasitoid larvae gut content indicated altogether the artificial diets requires the addition of others sources of proteins and lipids to improve the overall nutrition quality of the *in vitro* rearing system for this ectoparasitoid.

KEY WORDS: Biological control, fatty acid, idiobiont, nutritional requirement, protein

Although *in vitro* development of endoparasitic species improved in the last decade, few attempts were successful in rearing ectoparasitic hymenopterans (Xie *et al.* 1989; Yazlovetsky *et al.* 1992; Magro & Parra 2002, 2004). Nevertheless, several constraints must be addressed before many of the parasitoid species shown to develop on an

artificial diet could be routinely produced in large scale to attend the requirements of applied biological control programs (Cõnsoli & Parra 2002). Development of an artificial rearing system for parasitoids requires more than the provision of a high quality artificial diet to the immatures, but also an overall understanding of the requirements

imposed by the evolutionary history of the host-parasitoid interactions (Cônsoi & Parra 1999). The importance of natural enemies, particularly parasitoids, as biological control agents has fostered dietetic studies for the development of artificial diets as a way to reduce production costs in mass rearing facilities (Li 1992; Cônsoi & Parra 1997, 1999; Grenier 1997).

*Bracon hebetor* Say is a gregarious ectoparasitoid and an important biological control agent of several lepidopteran stored product pests due to its rapid growth and development rates (Keever *et al.* 1985, Prozell & Schöller 1998, Baker & Fabrick 2000). As an attempt to develop an alternative system to mass rear this parasitoid, an artificial diet was devised which yielded 40% of adults as compared to the 70% obtained when *B. hebetor* was reared on the natural host, which were similar in size and displayed the same parasitism and host handling behavior (Magro & Parra 2004). Besides the lower larval survivorship, 60% of the *in vitro*-reared pupae lacked the protective cocoon, which was hypothesized to be due to the low protein content of the artificial diet.

Therefore, we aim to understand the immature biology of *B. hebetor* and its nutrient acquisition behavior, and to detect proteins and lipids in the insect's digestive tract, based on comparative histochemical studies of *in vitro* and *in vivo*-reared larvae (following Magro & Parra 2004). This is a decisive step to understand both the nutritional benefits of this parasitoid's natural diet as means to improve its artificial diet, and to use the information as a model for rearing other parasitoids.

## Materials and Methods

### Maintenance of a stock culture of *B. hebetor*.

Ectoparasitoid couples were individually placed in cylindrical glass vials (2.0 cm diameter x 4.5 cm height) with rubber lids, and maintained on its natural host, *A. kuehniella*. After 24h, parasitized larvae were removed from the vials, transferred to petri dishes (6 x 2 cm), and kept under controlled conditions (25 ± 2°C, 70 ± 10% RH, and 14h photophase), until adult emergence. Part of the eggs obtained from parasitized *A. kuehniella* were transferred to the artificial diet according to Magro & Parra (2004).

**Artificial diet.** The diet was prepared with *Diatraea saccharalis* (F.) (Lepidoptera: Pyralidae) pupal holotissues (40%), egg yolk (20%), low-fat milk (20%), distilled water (10%) and a mixture of Neisenheimer's salt (10%), and 0,2% (v/v) antibiotic-antimycotic solution (Gibco- Brl).

Pupal holotissues were collected from 24h- to 48h-old pupae of *D. saccharalis* reared on an artificial diet (Parra & Mishfeldt 1992). Pupae were treated in a water bath at 60-62°C for 10 min to avoid hemolymph melanization (Shapiro & Ignoffo 1973), surface-sterilized in 2% sodium hypochlorite solution (10 min), and washed twice in sterile distilled water. Pupae were then placed in a 15 ml disposable syringe barrel and squeezed by pushing the plunger down. The holotissues macerate was collected into an appropriate container, centrifuged (4000 rpm x 3 min), and the

supernatant collected and stored at -18°C for further use. All steps for diet preparation and manipulation were conducted in a laminar flow hood to provide for the required asepsis (Magro & Parra 2004).

**Protein quantification.** Actively feeding 5<sup>th</sup>-instar larvae of *A. kuehniella* were ice-immobilized, a pair of the abdominal legs was severed and the exsuding hemolymph collected with the aid of a syringe was transferred into a 1.5 ml vial containing few crystals of 1-phenyl-2-thiourea (PTC) to avoid melanization. Three 500 µl pooled hemolymph samples were collected and used along with diet samples to test for the protein content according to Bradford (1976), using egg albumin as a standard).

**Development of *B. hebetor* immature stages.** The rearing medium (150 µl) was placed between stretched sheets of parafilm laid on top of a plastic petri dishes (2 x 1 cm), and 25 *B. hebetor* eggs obtained from parasitized *A. kuehniella* (4h maximum exposure time) were carefully transferred to the top parafilm layer. The rearing units were placed in an incubator (25 ± 2°C; 70 ± 10% RH; 14h photophase), and parasitoid development was daily monitored. Experiments consisted of ten replicates (25 eggs/diet container = one replicate). For comparison, all parameters were simultaneously evaluated for parasitoids reared on natural host (*A. kuehniella*) (control).

Eating activity and silk production were evaluated every 4h to determine duration of the larval and pupal stages, and of the total cycle. Mean duration of developmental time and total cycle were compared by the *t* test (P < 0.05).

### Food intake during larval development of *B. hebetor*.

Artificial diet was prepared following that previously described, in which 100.0 µl (10%) of 400 ppm "Amarante" coloring stock solution were added instead of distilled water {Amarante Dye – 3-hidroxi-4-[(4-sulfo-1-naphthalenyl)azo]-2,7-naphthalenedissulfonic acid trissodium salt}. This new diet was offered to *B. hebetor* to evaluate parasitoid larval feeding.

Parasitoids were placed to develop under controlled conditions (25 ± 2°C; 70 ± 10% RH; and 14h photophase), and three replicates of 15 larvae each for the *in vitro*- and *in vivo*-reared insects with and without coloring were taken at the end of the larval stage to determine the volume of the diet ingested by reading the absorbance at 523 nm in a Spectra Max Plus photometer. A previously defined standard curve for several diet concentrations with and without Amarante enabled the correlation of absorbance and diet volume. Samples of the diet without coloring were used as blanks.

**Larval development of *B. hebetor*.** To assess the immature development of *B. hebetor*, 5<sup>th</sup>-instar *A. kuehniella* larvae were offered to female parasitoids for 4h. After the parasitization period, females were removed and part of the eggs laid were transferred to the artificial diet, while the remaining were maintained on the natural host for further evaluation. *In vivo*- and *in vitro*-reared larvae were first

sampled 6h after hatching (50 larvae and three replicates for each sample of both treatment and every 12h afterwards). Samples were weighed on an analytical scale, fixed in 70% ethanol for at least 24h, placed in phenol for 6h, and mounted on slides on Hoyer's medium. Parasitoid larval mandibles were measured using a micrometer attached to a phase-contrast microscope to assess parasitoid growth and development by using the Dyar's rule (Parra & Haddad 1989, Cónsoli & Vinson 2002).

**Histochemical tests of the gut content of *in vivo*- and *in vitro*-reared *B. hebetor*.** The tests were conducted following the Aneline Blue Black technique for protein contrast, and the Sudan IV technique for lipid contrast (Fisher 1968, Johansen 1940). Guts of *B. hebetor* larvae reared on the artificial diet and on the natural host were removed at the end of the feeding stage (85-90h and 70-80h, respectively) and fixed in Karnovsky for at least 2h. Samples were dehydrated through an ethanol series (30%, 50%, 70%, 90%, and 100% - 10 min each). All samples were kept in historesin + ethanol for the first 2h, then in pre-infiltration resin overnight, and last in 100% resin. Three- $\mu$ m thick sections were cut with a steel razor, transferred on glass slides, stained and viewed on a light microscope.

## Results and Discussion

**Development of immature stages.** Larval and pupal development of *B. hebetor* reared on artificial diet were delayed in comparison to those reared on natural host (*A. kuehniella*) (Table 1). *In vivo*-reared insects (natural diet) stopped feeding and abandoned the natural host 80-84h after oviposition and started silk production (Fig. 1a) with the first threads being observed after only 8h (88-92h) (Fig. 1b). Silk production continued until 112-116h; from then on, the insect body started to differentiate thorax and abdomen. Larvae purged their gut contents (meconium - excrement accumulated in the digestive tract during larval development) approximately at 120h (Fig. 1c).

*In vitro*-reared insects (artificial diet) stopped feeding at 88-92h (Fig. 1e), 8h later than those reared on the host. The silk production was observed after 128-132h, although scattered and intermittent, covered some of the surface of rearing substrate (Fig. 1f). The purge of the gut occurred 56h after interruption of food intake; i.e. 16h later (84-124h) than it occurred for the larvae reared on the host (Fig. 1g).

Silk production occurred between 84h and 112h for insects reared *in vivo*, and between 96h and 140h for those

on artificial diet. In both cases, the gut was purged 8h after the end of feeding activity.

Using the purge of the gut as a parameter, larvae reared on artificial diet had the development arrested for 24h (total duration of 144-148h); for insects on natural host, total larval development took from 120h to 124h (Fig. 1c, g).

The parasitoid molted to the pupal stage soon after the purge of the gut, and pupal development was 1.25 day longer for insects reared on the artificial diet as compared to those reared on the natural host. Adult emergence occurred 246h after oviposition (126h duration), or 5.25 days (124-246h) for *in vitro*-reared insects and at 156h, or 6.5 days (144-304h) for those reared on *A. kuehniella* (Figs. 1d, h). Magro & Parra (2004) observed an increase in *B. hebetor* larval stage when insects grew on artificial diet, compared to insects on natural host. Similar data concerning the duration of this parasitoid's larval phase were obtained from insects reared on natural host (Magro & Parra 2001).

**Larval instars.** Both *in vivo* and *in vitro*-reared parasitoids showed three instars (Table 2), but 1<sup>st</sup> and 3<sup>rd</sup> instars development on the artificial diet were much longer (Figs. 2 and 3). The arrest in the development of both instars led to the overall delay in the larval development observed for *in vitro*-reared parasitoids. Insects reared on artificial diet underwent a 12h elongation period in the 1<sup>st</sup> instar, whereas for those on natural host it was 24h. The 2<sup>nd</sup> instar started 36h after hatching and lasted 12h (Fig. 3). On artificial diet, 3<sup>rd</sup> instar started 48h after eclosion and larvae abandoned the diet after 84h (mean duration of 36h). On the natural host, larvae initiated the 3<sup>rd</sup> instar 36h after eclosion and started moving away from the host 60h after eclosion, a 24h process.

**Food intake of *B. hebetor*.** Insects reared on natural host gained weight faster than on artificial diet. Twenty-four hours after eclosion, *in vivo*-reared insects already had 6.7% of their final weight whereas those *in vitro* had 1.7%. Insects on natural host gained most of their weight between 36h and 48h, when they went from 25% to 96% of their final weight. Their larval stage was completed 60h after eclosion, with larvae weighing 2.25 mg. The larval weight gain of *in vitro*-reared insects was similar to the *in vivo*-reared ones, but food intake was prolonged. These insects went from 18% to 86% of their final weight, at 36h and 60h after eclosion, respectively. Insects on artificial diet reached their maximum weight (2.93 mg) 72h after eclosion; 12h later, when they stopped feeding, they lost up to 5% of their total weight

Table 1. Duration (days) of egg, larval and pupal stages, and total cycle of *B. hebetor* reared on artificial diet and on the host *A. kuehniella* (25  $\pm$  2°C temperature, 70  $\pm$  10% RH, 14h photophase).

Treatments	Duration (days)			
	Egg	Larva	Pupa	Total cycle
Host	1.5 $\pm$ 0.01 a	4.8 $\pm$ 0.10 b	5.4 $\pm$ 0.04 b	11.0 $\pm$ 0.82 b
Diet	1.6 $\pm$ 0.01 a	5.8 $\pm$ 0.10 a	6.6 $\pm$ 0.11 a	13.3 $\pm$ 0.22 a

Means compared by *t* test,  $P \leq 0.05$ .

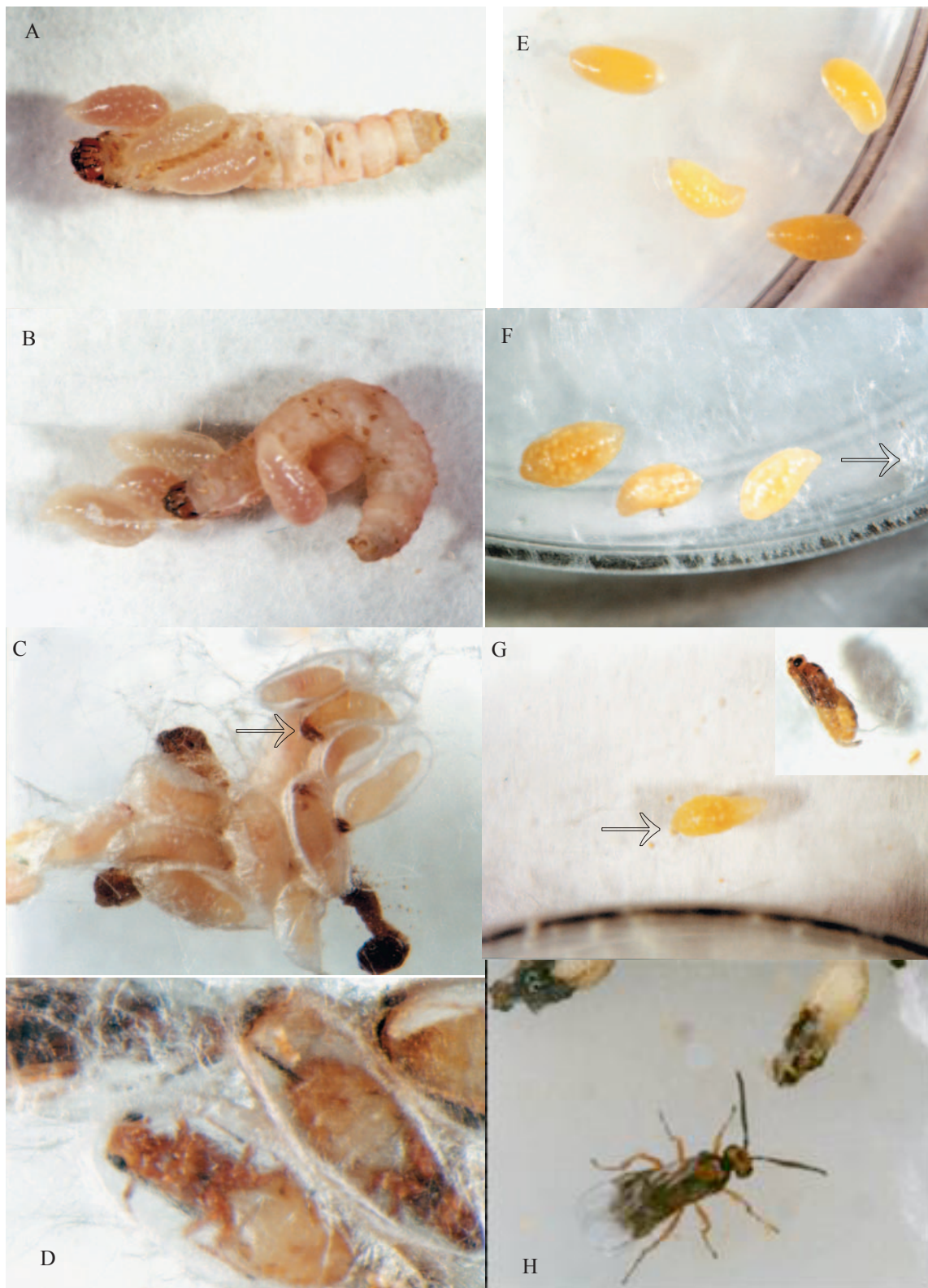


Fig. 1. *B. hebetor* development ( $25 \pm 2^\circ\text{C}$  temperature,  $70 \pm 10\%$  RH, 14h photophase). *In vivo*: A) 80-84 development hours (5-fold increase); B) 88-92h (5-fold increase); C) 116-120h (arrow indicates meconium) (5-fold increase); D) 246h – newly emerged adults (12-fold increase). *In vitro*: E) 88-92h (5-fold increase); F) 128-132h (arrow indicates silk) (5-fold increase); G) 144-148h (arrow indicates meconium) (naked pupa – 246h) (5-fold increase); and H) 304h – emergence of adults (8-fold increase).

Table 2. Mandible length (interval variation) and growth ratio of *B. hebetor* reared on natural host and artificial diets (25 ± 2°C; 70 ± 10% RH; 14h photophase).

Instars	Natural host		Artificial diet	
	Mandible length(μm)	Ratio	Mandible length (μm)	Ratio
1	29.5 ± 0.58 (20.8 – 36.3)	-	30.80 ± 0.65 (26.6 – 36.9)	-
2	50.4 ± 0.84 (47.2 – 55.5)	1.71	41.9 ± 0.63 (38.7 – 45.7)	1.33
3	61.9 ± 0.99 (56.4 – 68.5)	1.59	60.6 ± 0.37 (55.8 – 66.5)	1.45
R <sup>2</sup>	100%		100%	



Fig. 2. Measurement of mandible length of *B. hebetor* for the assessment of larval development (detail indicates measures in μm).

(Fig. 4). These insects were heavier than those on natural host. The prolonged development observed on the artificial diet indicates the unsuitability of the diet and low adaptation of the insect to this alternative nutrient resource.

In spite of the extension of the larval stage, the use of food coloring did not affect *B. hebetor* development. Based on the values of absorbance obtained, larvae took up 3.77 μl or the artificial medium, a 1.9-fold the final gut volume (2 μl) after 84h of continuous growth (from newly-eclosed to the beginning of the pre pupae).

By comparing Amarante consumption (all coloring retained in insects' digestive tube) to the larval final weight (2.93 mg), 20% of the food up taken was lost due to transpiration. Weight cannot be lost through feces because Hymenoptera parasitoids have blind gut and excrements

(meconium) are eliminated only at the onset of the pupal stage or at adult emergence.

Our data indicate the ideal dietetic volume for different numbers of "inoculated" eggs on artificial diets, and demonstrate that host-reared insects feeding for 60 consecutive hours on 5<sup>th</sup>-instar *A. kuehniella* larvae take the most advantage of the diet, considering food flow and timing because they ingest 2.69 μl hemolymph (2.25 mg weight). Besides, *A. kuehniella* had a higher concentration of protein (391.3 μg) than the artificial diet (108.9 μg). Protein deficiency may affect parasitoid's development by extending its egg-adult cycle.

Results also show that extension of the larval phase occurs at the 1<sup>st</sup> instar and at the end of the 3<sup>rd</sup>, at times when insects stop feeding and start silk production for cocoon formation, which takes a whole day. During the pupal stage,

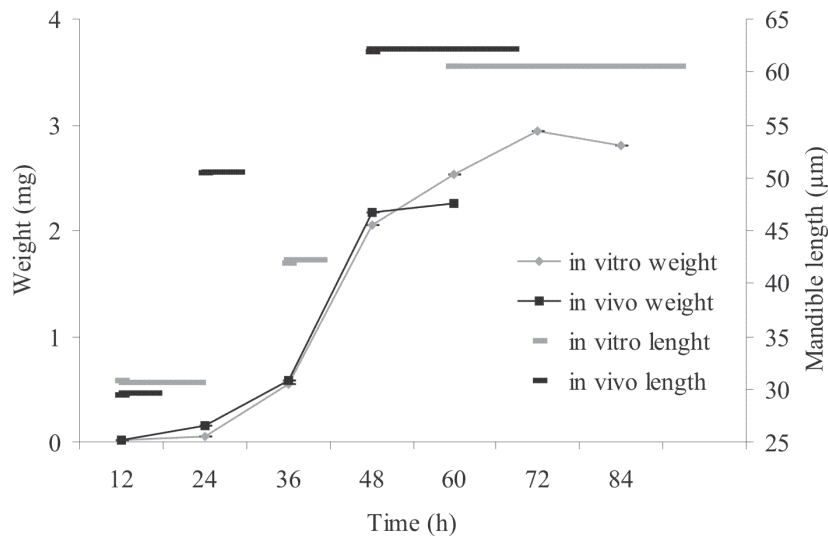


Fig. 3. Total weight gain (mg) during larval development and mandible length (arrows indicate number and duration of instars) of *B. hebetor* reared on artificial diet (*in vitro*) and on host (*in vivo*), in different sampling times (25 ± 2°C temperature, 70 ± 10% RH, 14h photophase).

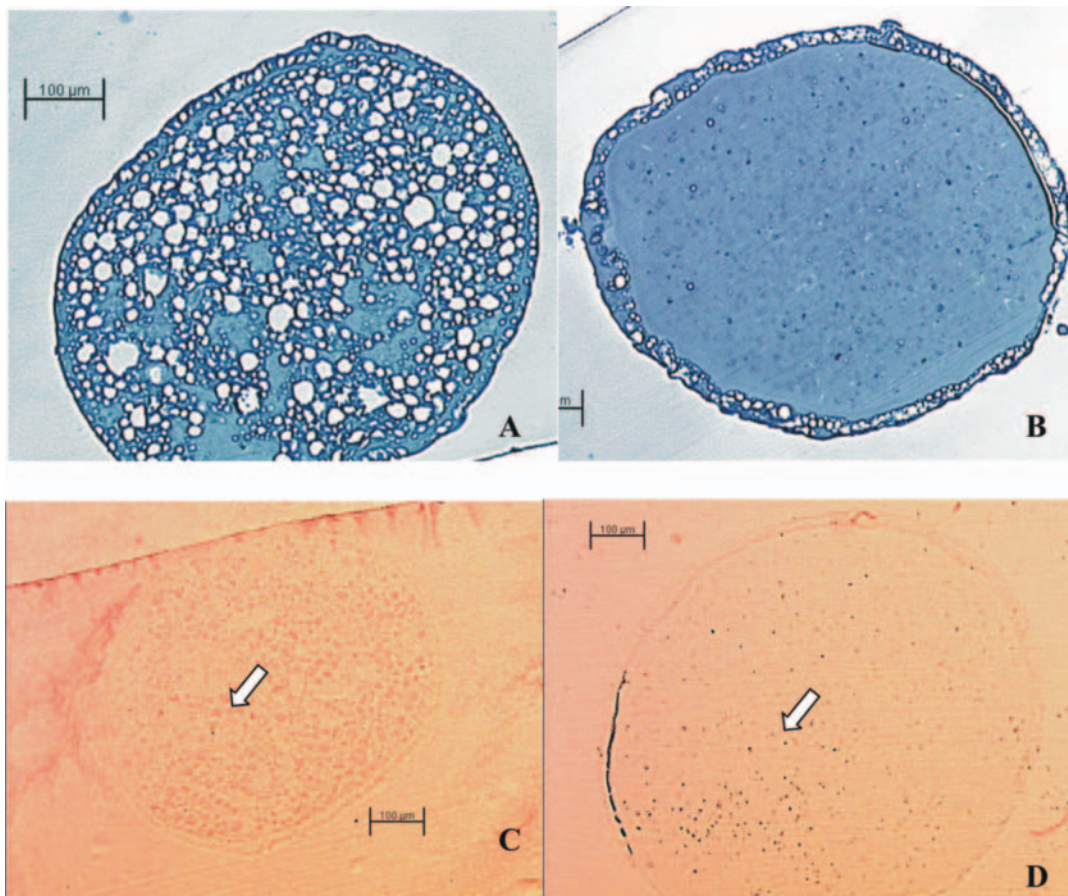


Fig. 4. Sections of midgut of *B. hebetor* reared *in vivo* (left) and *in vitro* (right). A and B – contrasted for proteins; C and D – contrasted for lipids. Arrows indicate lipid vesicles.

there is an average extension of 1.25 day. Therefore, cycle duration can be associated with artificial diet quality. Extension of the larval stage and consequently, longer ingestion period indicates a poor diet.

Insect growth, development and reproduction are positively correlated to the amount and quality of the ingested food. Therefore, both the amount and the quality of the food consumed during the larval stage affect growth rate, development, body weight, survival rate, as well as adult fitness (Parra 1991).

Parasitoids and predators follow House's (1961) nutritional requirements principles, and are qualitatively similar to those insects belonging to other orders, requiring for primary nutrients such as carbohydrates, lipids (including sterols), vitamins, and mineral salts, but these entomophagous insects have a special need for high-content protein diets (Dadd 1985, Parra 1991, Grenier 1997).

**Histochemistry of the digestive system of *in vivo*- and *in vitro*-reared *B. hebetor*.** Insects reared on artificial diet contained thinner and less granulated material, and small droplets (Fig. 4b). On the other hand, insects feeding on the natural host had larger droplets in the gut lumen (Fig. 4a). Sections contrasted for protein and lipids showed a higher

lipid concentration in the gut of larvae reared on the natural host (Fig. 4c), and a higher protein concentration (Fig. 4b) and small lipid droplets (Fig. 4d) in the gut of *in vitro*-reared larvae.

For Canavoso *et al.* (2001), most of the insects demand diets that are rich in poli-unsaturated fatty acids. They also reported that, according to several authors, both the linolenic and the linoleic acids adequately satisfy such need. However, demands for essential fatty acids may differ significantly among species. Parasitic Hymenoptera obtain fatty acids by ingesting their hosts. The koinobiont larval ectoparasitoid *Euplectrus separatae* (Hymenoptera: Eulophidae) parasitizes *Pseudaletia separate* Walker (Lepidoptera: Noctuidae), and grows by ingesting its hemolymph, which contains high lipid concentrations (Nakamatsu & Tanaka 2003, 2004). Norullahoğlu *et al.* (2004) studied the lipid content of the host and its koinobiont parasitoid *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae), and found that total fatty acid content in the parasitoid's larvae was significantly greater than in the host caterpillars (parasitized or not). In this case, host envenomation did not increase the ratio of lipids in the host hemolymph, and the palmitic, palmitoleic, stearic, oleic, linoleic, and linolenic acids represented 68% of all fatty acids in *Achroia grisella* (F.)

(Lepidoptera: Pyralidae), the *A. galleriae* host.

Therefore, the low protein-concentration of the diet (108.9 µg/µl) compared to the 391.3 µg in the host, resulted in the prolonged larval development of *B. hebetor*, longer ingestion time, and a larger food intake. Three larval instars for the development of the parasitoid were found in both rearing systems. Protein quantification and the histochemical tests corroborate the importance of proteic and lipidic components for the development of the larval ectoparasitoid *B. hebetor* in artificial diets, in an attempt to approximate the *in vitro* system to the system represented by the host larvae (*in vivo*). Therefore, addition of both protein and lipid sources such as casein, soroalbumin, and the linoleic and linolenic acids to the current artificial diet can improve the quality of *in vitro*-reared *B. hebetor*.

### Acknowledgments

Thanks are due to Beatriz A. da Glória, Departamento de Ciências Biológicas (ESALQ/USP), and Elliot W. Kitajima and Francisco Tanaka, Núcleo de Apoio à Pesquisa – Microscopia Eletrônica Aplicada à Agronomia (NAP-MEPA, ESALQ/USP), for helping with the histochemical analysis. To the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for funding this research, and to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the fellowship to the W. R. Terra.

### References

- Baker, J.E. & J.A. Fabrick. 2000. Host hemolymph proteins and protein digestion in larval *Habrobracon hebetor* (Hymenoptera: Braconidae). *Insect Biochem. Mol. Biol.* 30: 937-946.
- Bradford, M.M. 1976. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248-154.
- Canavoso, L.E., Z.E. Jouni, K.J. Karnas, J.E. Pennington & M.A. Wells. 2001. Fat metabolism in insects. *Ann. Rev. Nutri.* 21: 23-46.
- Cônsoli, F.L. & B. Vinson. 2002. Larval development and feeding behavior of the wing dimorphics of *Melittobia digitata* Dahms (Hymenoptera: Eulophidae). *J. Hym. Research.* 11:188-196.
- Cônsoli, F.L. & J.R.P. Parra. 1997. Produção 'in vitro' de parasitóides: criação de *Trichogramma galloi* e *T. pretiosum* no Brasil, p.259-302. In J.R.P. Parra & R.A. Zucchi (Eds.), *Trichogramma e o controle biológico aplicado*. Piracicaba, FEALQ, 324p.
- Cônsoli, F.L. & J.R.P. Parra. 1999. *In vitro* rearing of parasitoids: Constraints and perspectives. *Trends Entomol.* 2: 19-32.
- Cônsoli, F.L. & J.R.P. Parra. 2002. Criação *in vitro* de parasitóides e predadores, p.249-271. In J.R.P. Parra, B.S. Corrêa-Ferreira, P.S.M. Botelho & J.M.S. Bento (eds.), *Controle biológico no Brasil, parasitóides e predadores*. Barueri, Manole, 609p.
- Dadd, R.H. 1985. Nutrition: Organisms, p.313-390. In G.A. Kerkut & L.I. Gilbert (eds.), *Comprehensive insect physiology biochemistry and pharmacology. Regulation: Digestion, nutrition, excretion*. Pergamon Press, 639p.
- Fisher, D.B. 1968. Protein staining of ribboned epon sections for light microscopy. *Histochemie* 16: 92-96.
- Grenier, S. 1997. Desenvolvimento e produção *in vitro* de *Trichogramma*, p.235-258. In J.R.P. Parra & R.A. Zucchi (eds.), *Trichogramma e o controle biológico aplicado*. Piracicaba, FEALQ, 324p.
- Guerra, A.A. 1992. *In vitro* rearing of *Bracon mellitor* and *Catolaccus grandis* with different insect hemolymph-based diets. *Southwest. Entomol.* 17: 123-126.
- House, H.L. 1961. Insect nutrition. *Annu. Rev. Entomol.* 6: 13-20.
- Johansen, D.A. 1940. Phant microtechnique. New York, McGraw-Hill, 108p.
- Keever, D.W., R.T. Arbogast & M.A. Mullen. 1985. Population trends and distributions of *Bracon hebetor* Say (Hymenoptera: Braconidae) and lepidopterous pests in commercially stored peanuts. *Environ. Entomol.* 14: 722-725.
- Li, L. 1992. *In vitro* rearing of parasitoids of insect pests in China. *Korean J. App. Entomol.* 31: 241-246.
- Magro, S.R. & J.R.P. Parra. 2001. Biologia do ectoparasitóide *Bracon hebetor* Say, 1857 (Hymenoptera: Braconidae) em sete espécies de lepidópteros. *Sci. Agric.* 58: 693-698.
- Magro, S.R. & J.R.P. Parra. 2002. Criação *in vitro* de *Bracon hebetor*, p. 277-293. In J.R.P. Parra, B.S. Corrêa-Ferreira, P.S.M. Botelho & J.M.S. Bento (Eds.), *Controle biológico no Brasil, parasitóides e predadores*. Barueri, Manole, 609p.
- Magro, S.R. & J.R.P. Parra. 2004. Comparison of artificial diets for rearing *Bracon hebetor* Say (Hymenoptera: Braconidae). *Biol. Control.* 29: 341-347.
- Nakamatsu, Y. & T. Tanaka. 2003. Venom of ectoparasitoid *Euplectrus sp* near *plathypenae* (Hymenoptera: Eulophidae) regulates the physiological state of *Pseudaletia separata* (Lepidoptera: Noctuidae) host as a food resource. *J. Insect Physiol.* 49: 149-159.
- Nakamatsu, Y. & T. Tanaka. 2004. Venom of *Euplectrus separatae* causes hyperlipidemia by lyses of host fat body cells. *J. Insect Physiol.* 50: 267-275.
- Norullahoğlu, Z.Ü., F. Uçkan, O. Sak & E. Ergin. 2004. Total lipid and fatty acid composition of *Apanteles galleriae* and its parasitized host. *Physiol. Biochem. Toxicol.* 9: 1000-1006.
- Parra, J.R.P. 1991. Consumo e utilização de alimentos por insetos, p.9-65. In A.R. Panizzi & J.R.P. Parra (eds.), *Ecologia nutricional de insetos e suas aplicações no manejo de pragas*. São Paulo/SP, CNPq, Ed. Manole,359p.
- Parra, J.R.P. & L.H. Mishfeldt. 1992. Comparison of artificial diets for rearing the sugarcane borer, p. 195-209. In T.E. Anderson & N.C. Leppla (eds.), *Advances in insect rearing for research and pest management*. Colorado, Westview Press, 517p.

- Parra J.R.P. & M.L. Haddad. 1989. Determinação do número de instares de insetos. Piracicaba/SP. Fealq, 49p.
- Prozell, S. & M. Schöller. 1998. Insect fauna of a bakery, processing organic grain and applying *Trichogramma evanescens* Westwood. Integrated Protection of Stored Products IOBC Bulletin. 21: 39-44.
- Shapiro, M. & C.M. Ignoffo. 1973. Growth of *Heliothis zea* (Lepidopera: Noctuidae) cells adapted to various culture media. Ann. Entomol. Soc. Am. 66: 270-273.
- Xie, Z., L. Li & Y. Xie. 1989. *In vitro* culture of *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae). Chin. J. Biol. Control. 5: 49-51.
- Yazlovetsky, I.G., A.M. Neponnyashaya & L.I. Ageeva. 1992. Optimization of the artificial nutritional diet for the larvae of *Bracon hebetor* (Hymenoptera, Braconidae). Zoo. Zh. 71: 134-138.

*Received 19/I/05. Accepted 31/V/05.*

---